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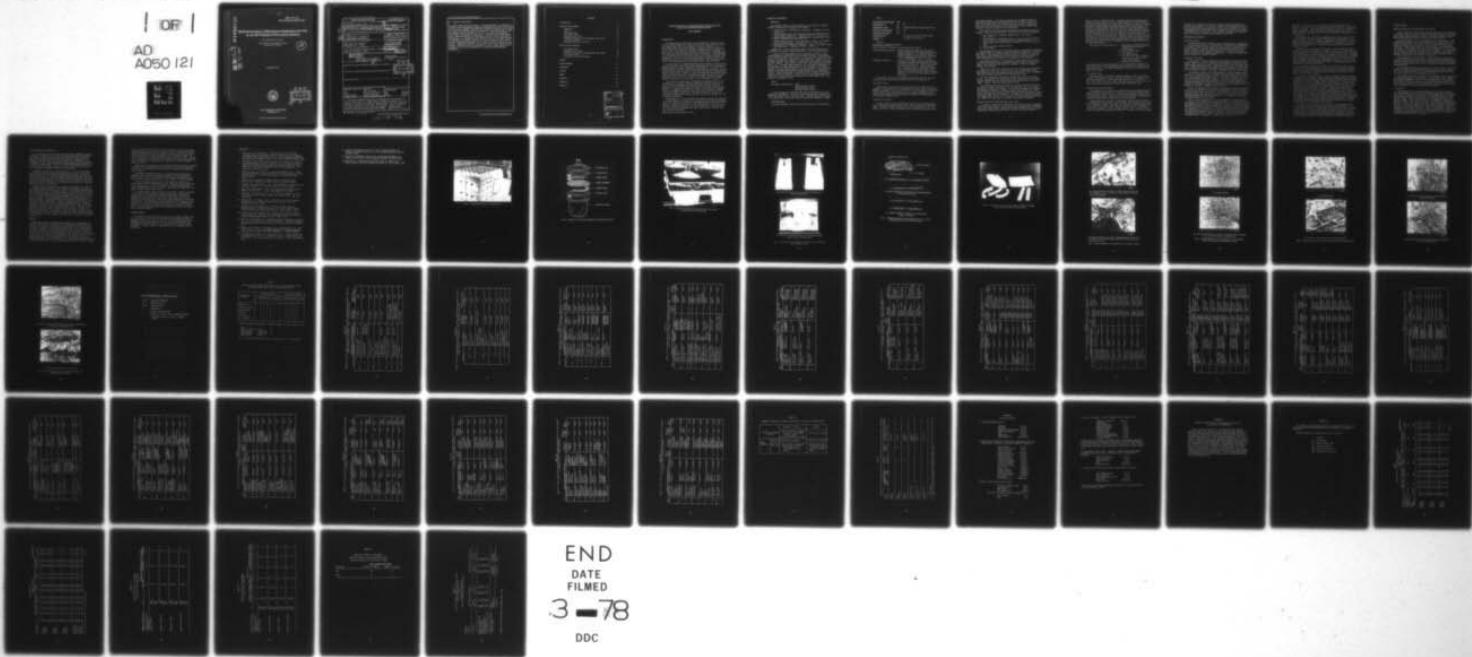
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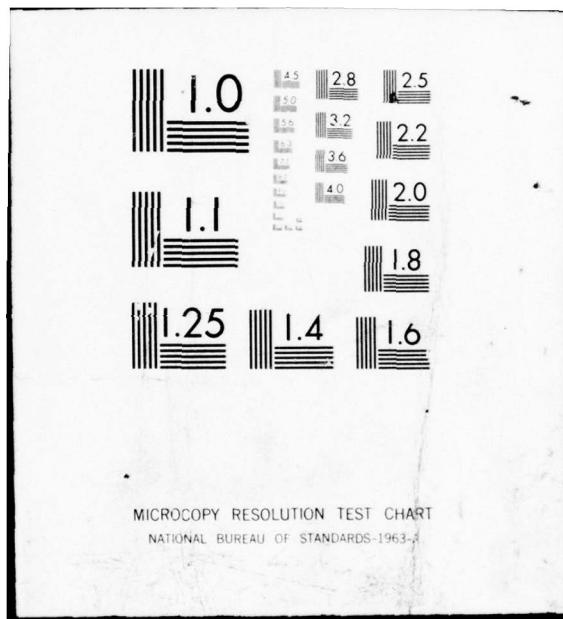
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## Biodeterioration of Membrane Separators for Use in an Oil Pollution Prevention System

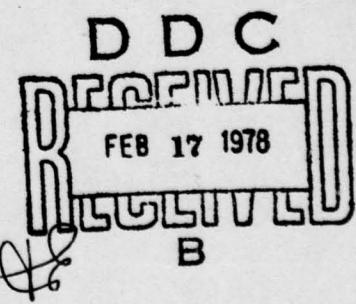
M. E. MAY and R. A. NEIHOF

Marine Biology and Biochemistry Branch  
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Prototype membrane materials for possible use as separators of ballast water and oil in cargo tanks of oilers and tanker ships (in a membrane oil pollution prevention system, MOPPS) have been evaluated for their resistance to microbial deterioration. Fabric reinforced elastomer composites of two types were tested: neoprene/nylon fabric/nitrile and Hydrin/nylon fabric/Hydrin. Samples with cold and		

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hot-formed seams were also tested. Following exposure in systems inoculated with microorganisms commonly associated with fuels for periods up to one year, membrane specimens were inspected visually and by light and electron microscopy and also subjected to standard physical tests. The changes observed appeared to be due to oil and water exposure rather than to microbial deterioration. Although the neoprene/nylon/nitrile membrane appeared a promising choice for further performance evaluations, it is essential that pinholes and open cut edges at seams be minimized if extensive diffusion of water and oil into the fabric and the accompanying weakening of the fabric-elastomer bond are to be avoided. Numerous microscopic cracks were noted in the exposed Hydrin surfaces indicating that the formulation of this rubber should be re-evaluated if it is to be retained as a possible backup membrane candidate.

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## BIODETERIORATION OF MEMBRANE SEPARATORS FOR USE IN AN OIL POLLUTION PREVENTION SYSTEM

### FINAL REPORT

#### INTRODUCTION

The background and objectives of the Membrane Oil Pollution Prevention System (MOPPS) for installation in the cargo tanks of oilers and tanker ships have been presented in detail elsewhere (1,2,3). Briefly described the system proposes to separate ballast water from oil or fuel in the tanks by a flexible membrane bag or diaphragm. A major source of marine pollution from oil entrained in discharged ballast water and tank washings would thus be eliminated. An improvement in the quality of the oil carried could also be expected because emulsification of water in the oil would be greatly reduced and the conditions known to lead to growth of fungal mats, fuel-souring bacteria, and to the proliferation of particulate matter would be denied.

A major part of the MOPPS program has been devoted to selection of membrane materials and installation configurations which would provide maximum service life and operational efficiency (3). In addition to having adequate strength, flexibility and abrasion resistance, the membrane should also be resistant to deterioration by the microorganisms present in the tanks. This is a realistic consideration because elastomeric and plastic materials are known to support the growth of microorganisms (4,5,6) and the existence of thriving cultures of fungi, yeasts, and bacteria in fuel tanks is well documented (7,8,9,10).

This study was undertaken to determine the susceptibility to deterioration of candidate membrane separator materials exposed to a variety of microorganisms selected from those typically growing in hydrocarbon fuel storage tanks under both aerobic and anaerobic conditions. It appeared advisable to include an evaluation of membrane seams since membrane installations of the size envisioned in MOPPS could not be made in one piece. The use of crude oil as well as different commonly used distillate fuels in the test systems was also considered necessary.

Unfortunately, it is not possible to perform accelerated exposures of biodeterioration on materials in the same way accelerated physical tests are carried out because living organisms have relatively limited ranges of conditions for growth. The only recourse is to expose the materials under investigation to active cultures of organisms under the most favorable conditions for their growth and to run the tests for as long as possible.

Note: Manuscript submitted November 18, 1977.

## MATERIALS AND METHODS

### Membranes:

The membrane materials used consisted of two layers of various synthetic rubbers with a nylon fabric between:

1. Chemivic/nylon cloth/Chemivic (Goodyear). Thickness 1.75 mm (0.069 in.).
2. Neoprene/nylon fabric with unidirectional thread/nitrile (Uniroyal Sealdrum material). Thickness 3.89 mm (0.153 in.).
3. Neoprene/nylon fabric with unidirectional thread/nitrile (Uniroyal) Thickness 3.56 mm (0.140 in.)
4. Neoprene/nylon cloth/nitrile (Uniroyal). Fabric - 13 oz/yd<sup>2</sup> nylon 2x2 basketweave. Thickness 2.21 mm (0.87 in.).
5. Hydrin/nylon cloth/Hydrin (Uniroyal). Fabric - 13 oz/yd<sup>2</sup> nylon 2x2 basketweave. Thickness 1.78 mm (0.070 in.).

Samples of 1, 2 and 3 were used only in the early phases of the work before the proper candidate materials, 4 and 5 were received.

In much of the experimental work it appeared desirable to use rubber membrane samples which were initially sterile. Four methods of sterilization were tried in order to find one which caused the least change in the physical properties of the materials. These were autoclaving, ethylene oxide exposure, washing with hypochlorite, and washing with ethanol. The results of this study were reported by May and Neihof (11). Although there appeared to be no marked differences in the properties of the membranes due to the different sterilization methods, the hypochlorite method was chosen because of its known efficiency in killing surface-borne microorganisms and its convenience (12,13). While autoclaving is no doubt the best method of attaining complete sterility, however, it was felt that this might change the cure state of the rubber and perhaps alter the susceptibility of the rubbers to microbial attack.

### Fuels:

The fuels studied were: JP-5  
Marine Diesel (D.F.M.)  
Navy Distillate (N.D.)  
Mid-East Crude (M.E.C.)

It was necessary to sterilize the above fuels before use in the subsequent experiments. The fuels were autoclaved for 45 minutes at 121°C and oven-dried for 1 hour at 110°C. The Mid-East Crude showed a 3% weight loss. The others showed insignificant changes in weight.

### Microorganisms:

The microorganisms used in the aerobic studies are listed below.

Fungi:

<u>Aureobasidium pullulans</u>	279C	Quartermaster Culture Collection
<u>Trichoderma</u> sp.	365	
<u>Aspergillus niger</u>	386	
<u>Penicillium funiculosum</u>	391	
<u>Chaetomium globosum</u>	495	
<u>Cladosporium</u> sp.		Isolates from JP-5 tank in NAS Lemoore, California
<u>Candida</u> sp.		

Bacteria:

Pseudomonas aeruginosa QMB 1468

Three isolates of naturally occurring marine bacteria.

Curtis Bay mixed culture - obtained from fuel tanks of naval ships being scrapped at Curtis Bay, Maryland. The inoculum consisted of a mixed culture of sulfate-reducers, other bacteria including a marine Pseudomonas, fungi and yeasts.

Anaerobic cultures - consisted of a mixed microbial population of sulfate-reducers and associated bacteria originally isolated from infected fuel tanks of an aircraft carrier. These organisms have been maintained in continuous culture (10). Actively growing subcultures were prepared in Sisler's medium, triple strength (Sisler's 3x) (10), for inoculation of the test units.

The formulae for the more complex growth media used for culture maintenance and viability evaluations are given in Appendix A.

Agar Plate Tests:

These tests were carried out to determine if the rubbers or the methods of sterilization were inhibitory to selected fungi and bacteria. Membrane specimens 1.27 cm x 12.7 cm ( $\frac{1}{2}$ " x 5") were placed on the surfaces of agar plates and sprayed with an aqueous suspension of the fungal spores and bacteria listed above according to ASTM designators G 21-70 and G 22-67T (14,15).

Two-Phase Test Units:

A major portion of the program was carried out in one-half gallon glass containers simulating fuel tanks. Each contained a rubber membrane, enriched seawater inoculated with microorganisms and an over-layer of fuel oil. These containers were covered by metal screw caps

with Teflon liners. Two kinds of systems were examined: aerobic in which the aqueous phase was equilibrated with air and anaerobic in which the aqueous phase was equilibrated with nitrogen. The enrichment of the sea water medium and the organisms in the inoculum also differed in the two systems as described below.

The apparatus for both aerobic and anaerobic systems was set up as follows: Into each pre-sterilized 1/2 gallon (1.89 l) jar was placed:

- 1 - The "sterilized" candidate membrane 20.3 cm x 30.5 cm (8" x 12").
- 2 - Two previously "sterilized" 20.3 cm x 2.54 cm (8" x 1") membrane strips (for seamless Hydrin and neoprene/nitrile).
- 3 - One sterile cold rolled steel rod 0.318 cm x 14.0 cm (1/8" x 5 1/2").
- 4 - 900 ml enriched seawater medium.
- 5 - 450 ml fuel.

The seawater medium for the aerobic system was prepared by filtering the needed amount of seawater using a Millipore Type HA filter (0.45  $\mu$  pore size). One part sterile distilled water was added to 1 part seawater and sterile peptone-yeast extract (Difco) was added to give a final concentration of 0.05% each of peptone and yeast extract.

The seawater medium for the anaerobic system consisted of 0.275% Trypticase Soy Broth extract (without dextrose) (BBL) in filtered seawater. This medium was deaerated by passing a stream of nitrogen through it for 20 min.

There was a total of 8 containers for each system, both inoculated and control for each fuel (JP-5, D.F.M., N.D., and Mid-East Crude). One exception was for the neoprene/nylon cloth/nitrile (No. 4) seamless which was studied using only D.F.M. and Mid-East Crude.

The test systems were shaken 15 seconds every hour on a rotary shaker. The systems were maintained at room temperature which varied between 22-30°C and were covered with black cotton fabric to exclude light. Figure 1 shows the experimental set-up.

The systems were monitored weekly for the first month followed by once every 2 months to ascertain if the organisms were still viable. To monitor the anaerobic system, 1 ml of the water phase was withdrawn and cultured in Sisler's 3x medium at 26°C under mineral oil. The aerobic systems were monitored using Tryptone-yeast-glucose agar (Difco-Plate Count Agar) for the bacteria. The fungi were cultured on Cooke Rose Bengal Agar (Difco) with an addition of tetracycline hydrochloride at a level of 35 mg/l agar, to inhibit the growth of bacteria.

#### Membrane Separated Two-Compartment Test Units:

In order to approach the conditions that would be met in a ballast tank, test units were set up with Hydrin and neoprene/nitrile membranes (with and without seams) separating a seawater phase from an oil phase. Large glass desiccators were used to build this system (Figure 2).

Figure 3 shows an assembled unit. Plexiglas rings with portholes and tubulations were used on each side of the membrane to allow complete filling and sample taking from each phase. Six units were set up using hot-seamed, cold-seamed, or seamless neoprene/nitrile or Hydrin. The offset in thickness produced by the overlap of the seamed sheets necessitated the making of 1 inch (2.54 cm) wide rubber gaskets from the same membrane material which fitted around the perimeter of the sheets in the non-seamed portion to make a leakproof junction between the flanges of the desiccator (Fig. 2). Sealing on the water side was done with General Electric RTV-102 white (silicone rubber) while the D.F.M. side was sealed with Sealit S-681 manufactured by Fisher Scientific Co. The units were set on a rack with magnetic stirrers underneath to provide periodic agitation.

The seawater side consisted of:

- 1 - 50% seawater filtered through a Millipore Type HA Filter (0.45 $\mu$  size)
- 2 - 50% distilled water
- 3 - 0.1% peptone
- 4 - 0.1% yeast extract
- 5 - 2.54 cm x 24.1 cm x 0.0793 cm (1" x 9.5" x 0.0312") cold-rolled steel strip
- 6 - magnetic stirring bar

The inoculum for the seawater phase (lower compartment) consisted of Curtis Bay mixed culture combined with the bacterial and fungal varieties listed above. The upper compartment of each unit was filled with Marine Diesel Fuel.

#### Soil Burial Tests:

Neoprene/nitrile and Hydrin (membrane samples 4 and 5) were subjected to soil burial testing. The test facility was located at the U.S. Army Natick Labs. The samples were cut in 12.7 cm x 20.3 cm (5" x 8"), 7.62 cm x 20.3 cm (3" x 8") and 2.54 cm x 15.2 cm (1" x 6") pieces and tested according to Method 5762, CCC-T-191b, 1953.

#### Scanning Electron Microscopy:

The membranes were examined for microflora using an AMR Model 1000 (American Metals Research Corp.) scanning electron microscope (SEM). Small rectangular samples were cut from rubber specimens taking care not to disturb the surface. These were air dried, mounted on a SEM holder and gold plated before examination with the microscope.

Micrographs were also made of the rubber surfaces after they had been cleaned in various ways. All specimens were scrubbed with a brush in a "Sparkleen" (Fisher Scientific Co.) solution, rinsed several times in tap water and given a final rinse in distilled water. Membranes which still appeared to have a surface deposit were treated with benzene to remove any remaining fuel residue. Of these, a few were further

treated with a dilute HCl solution to remove corrosion products deposited on the surface. In order to improve the visualization of surface cracks, samples 3 cm long and 0.5 cm wide were mounted in a bent configuration in an SEM holder which confined the ends of the specimen to a distance of 1.3 cm apart. The procedure resembled that used by Bascom in a study of rubber tearing under tension (16).

#### RESULTS AND DISCUSSION

##### Agar Plate Tests:

The inoculated rubber membranes on the agar plates were observed at 1, 2, and 6 week intervals. Any evidence of inhibition or stimulation of microbial growth on and around the rubber strips was noted and is given in Table 1. Moderate inhibition of growth was observed in the presence of the rubber specimens compared to a control with filter paper. There were no significant differences in inhibition among samples sterilized by different methods.

##### Two-Phase Test Units:

In the aerobic systems during the early phases of the work, using rubber samples 1, 2, and 3, (Materials and Methods) monitoring of the aqueous phase showed an initial logarithmic growth rate the first week after inoculation followed by a leveling off for about two months and then a gradual decline in the number of living organisms. The die-off was not complete, however, and viable organisms could always be found.

In anaerobic systems with neoprene/nitrile (unidirectional fabric) material, it was found that the sulfate-reducing bacteria did not survive one week under the D.F.M. and N.D., only one week under the JP-5, and 3 weeks under the Mid-East Crude. The sulfate-reducers grew well in all of the controls with no rubber. This led to experiments to determine whether inhibition and/or kill of the sulfate-reducers was caused by the method of rubber sterilization or by the rubber itself (11). Appendix B contains a summary of these results.

Since this problem occurred only in the neoprene/nitrile (unidirectional fabric) and not in the candidate membranes, it was necessary to revitalize the former system only. Revitalization consisted of siphoning the seawater out from under the fuel and replacing it with fresh deaerated seawater-Trypticase Soy Broth. A new inoculum of sulfate-reducing bacteria was also added.

After 6 months incubation, one of the membrane strips was removed from each of the sample bottles containing them. The remaining strips and membranes were allowed to incubate for one year. Upon removal, the membranes were given a visual examination. Tables 2 through 10 tabulate the results of this examination. Surface deposits were noted, examined microscopically, and cultured on both Tryptone-yeast-glucose agar (TYG) and Potato-dextrose agar plus yeast (PDA+Y). Observations of changes in adhesion of seams or rubber to fabric and in surface appearance (becoming tender, roughened or losing surface lustre) are included.

Anaerobic test units were cultured for active sulfate-reducers on Sisler's 3x media, as well as for growth on TGY and PDA+Y. In certain cases, for example, Table 8b and 10b, there was little or no growth of aerobic organisms. This means that the culture media used did not select for the organisms present, and/or the dominant organisms present were anaerobic.

Photographs were taken of the membranes upon removal from the test units. Figure 4 shows the typical appearance of the membranes. The neoprene tended to swell causing the membranes to curl with the nitrile rubber on the concave side. There was also a heavy deposit across the center of the membranes at the position of the interface between the fuel and seawater.

Table 11 gives a summary of the results of the examinations for adhesion of seams and of rubber to fabric. The "fair to poor" adhesion of the neoprene to the fabric in the fuel phase appears to be one result of the "softening or tenderizing" of the neoprene by the fuel. Although the actual installation in a shipboard fuel tank would have the neoprene in the seawater compartment of a tank and the nitrile on the fuel side, there is enough wicking through seam edges and surface pin holes into the nylon fabric that adhesion could become a major problem unless this penetration is somehow eliminated.

In the Hydrin studies, the finish on the rubber was dulled and in some cases appeared roughened, but there was no "softening or tenderizing" as seen with the neoprene. The surfaces were further examined with the scanning electron microscope and these observations will be dealt with in a later section.

The results of physical tests, tensile strength, modulus, ultimate elongation, tear strength, strain energy and elastomer-to-fabric bond as obtained by the Naval Ship Research and Development Center (DTNSRDC), Annapolis, are given in Tables I and II, Appendix C. According to an analysis of the data by DTNSRDC there were no significant differences between physical properties of the membrane materials exposed in the inoculated and uninoculated test units. There were obvious and expected differences between the physical properties of the original unexposed materials and those of the exposed materials due to the contact with oil and water. The adhesion of neoprene and nylon fabric, for example, is significantly less due to swelling and softening in the oils (Table 11). It should be kept in mind that the tensile properties are largely those of the nylon fabric and not the rubbers. Electron microscopic examination can give additional insight into the changes taking place in the elastomers (see below).

It should be pointed out that in no case were the uninoculated control test units entirely free of microbial contamination. It is very difficult, short of autoclaving, to sterilize the inner filaments of the fabric in the membranes. Where seawater and nutrients are absorbed into the fabric, microorganisms can proliferate. For this reason rigorous comparison of the properties of membranes exposed to microorganisms with those exposed to sterile oil and seawater alone

cannot be made.

**Membrane-Separated, Two-Compartment Test Units:**

In less than four weeks in the membrane-separated, two-compartment test units there was a rapid change to anaerobic conditions as noted by the generation of ferrous sulfide in the aqueous phase. Figure 3 shows the blackening inside the desiccators after one year.

Droplets of water or oil were observed on the rubber tabs of the seamed discs extending out of the desiccators as illustrated in the diagram in Figure 5a. This results from the outward diffusion of liquid entering the nylon fabric at the raw edge of the seam inside the units. A very small amount of seawater could also be seen in the fuel phases of the Hydrin, cold and hot seam samples. It is important to note this in any future considerations of membrane seam design because, unless engineered properly, there could be seepage (Fig. 5b). Possible solutions are illustrated in Figure 5c.

Upon dismantling the desiccators after one year of incubation, both the fuel and the seawater phases were tested for hydrogen sulfide production using lead acetate paper. Hydrogen sulfide was present in all the seawater phases (Table 12) and in the fuel phase of the Hydrin, seamless sample.

Active sulfate-reducers were present in all test units. Bacteria, yeasts and fungi were subcultured from the seamless neoprene/nitrile test unit; bacteria and yeast from the Hydrin, hot seam, test unit; and only bacteria from the remaining test units.

David Taylor Naval Ship Research and Development Center (Annapolis) tested these membranes for elastomer - fabric bond. Their results are given in Appendix C, Table III. When compared with the adhesion of the fabric to the elastomer in the untreated sample in Table II, there does not appear to be any differences. Unfortunately, the data are too limited to make any conclusive statements.

**Soil Burial:**

Upon removal of the candidate membranes after 9 months burial, both the neoprene/nitrile and Hydrin membranes appeared to have supported scattered sparse fungal growth. The neoprene/nitrile samples were somewhat curled while there were no noticeable changes in the Hydrin. Figure 6 shows the appearance of the membranes upon their receipt at NRL. These samples were sent to DTNSRDC in Annapolis for physical testing. The results of these tests are given in Appendix C, Table IV. It was noted that there was a slight increase in the apparent cure state of the rubber but no significant practical changes compared to the original material. This is a significant result since resistance to the wide variety of microorganisms present in soil may be taken as a good indication of low susceptibility of a material to biodeteriorative processes in general.

#### Scanning Electron Microscopy:

In the studies of the microflora on the rubber membranes, typical membranes were chosen and a series of scanning electron micrographs were made. Figures 7a and 7b show bacterial and fungal growth on Hydrin rubber (hot seam) inoculated with aerobic organisms in the seawater - JP-5 system, and cold seam Hydrin in an anaerobic system of seawater - N.D. Figure 7b shows a mass of bacteria at the interface of the fuel and seawater. This is the microscopic appearance of a "deposit at the interface" given as a visual description in Tables 2 - 10. In Figure 7a fungal material can also be seen.

Figures 8a and 8b show the neoprene side of the neoprene/fabric/nitrile membrane before and after exposure for one year in the Two-Phase System. The original and exposed Hydrin surfaces are shown in Figures 9a and 9b. It should be noted that the Hydrin membrane shows more extensive surface cracks and flakes than the neoprene. An analysis of the surface deposits on the Hydrin rubber was made using x-ray energy dispersive spectrometry. The analysis indicated zinc, lead and iron were present in the flakes.

Because the Hydrin showed this flaking and cracking, its surface was studied more extensively. Two series of micrographs were made of the original Hydrin and the Hydrin exposed to the seawater-fuel system after treatment with a dilute hydrochloric acid solution and with benzene. These micrographs are shown in Figures 10a, b, c and d. As can be seen in Figure 10b, treating the membrane with benzene did not remove the flakes. Further treatment with the dilute HCl solution removed the flakes and exposed underlying cracks which appeared to be in the rubber itself (Figure 10c). Thus it appeared that the flakes were a mixture of extracted rubber compounding ingredients and corrosion products from the steel strip in the test unit since they are at least partially metallic and were removed by dilute acid. A typical crack exposed in the rubber after benzene and HCl treatment is shown at higher magnification in Figure 10d. Under the slight tension produced by bending the specimen it can be seen that the crack penetrates deeply into the rubber. No such cracks were noted in the neoprene or nitrile surfaces.

#### SUMMARY

Prototype membrane materials for possible use as separators for ballast water and oil in cargo tanks of oilers and tanker ships (in a membrane oil pollution prevention system, MOPPS) have been evaluated for their resistance to microbial deterioration. The microbiological exposures consisted of (1) determination of inhibition or stimulation of growth of surface-deposited microorganisms, (2) one-year exposure in two-phase test units with different oil and seawater media inoculated with aerobic and anaerobic microorganisms, (3) one-year exposure in a membrane-separated, two-compartment system inoculated with a mixed microbial culture, and (4) soil burial. Two principal membrane materials were tested: one with a layer of neoprene and a layer of nitrile rubber

with nylon fabric between and another with two layers of an epichlor-hydrin rubber (Hydrin) with nylon fabric between. Specimens with overlap seams bonded by cold and hot-formed processes were also evaluated. The oils used in the various exposures were (1) Mid-East Crude, (2) Diesel Fuel Marine, (3) Navy Distillate and (4) JP-5 jet fuel. Following the exposures the membranes were inspected visually and by scanning electron microscopy. Specimens were subjected to various standard physical tests.

There was no evidence that the candidate membrane materials inhibited the growth of microorganisms on their surfaces. An observed deterioration of the neoprene-fabric bond could be attributed to exposure to the oil phase.

According to DTNSRDC there was no consistent evidence that the physical properties of the membranes suffered from microbiological deterioration. The deterioration which was observed appeared due to oil and water exposure rather than to the presence of microorganisms.

In the two-compartment systems it was found that both oil and water could diffuse considerable distances along the nylon fabric from a cut edge at a seam. This could lead to a weakened rubber/fabric bond and emphasizes a need to eliminate open edges and pinholes in the rubber surfaces during fabrication.

On exposed Hydrin rubber surfaces the presence of many fine cracks was observed with the scanning electron microscope. Although the rubber faces of the membranes contribute relatively little to the strength of the membrane it would appear likely that these cracks could easily grow into holes under the flexing conditions of an actual installation. The formulation of this rubber may thus need to be re-evaluated if it should later be necessary to use it in a MOPPS installation. On the basis of information available at present it would appear that the neoprene/nylon/nitrile combination is the best choice for a candidate material.

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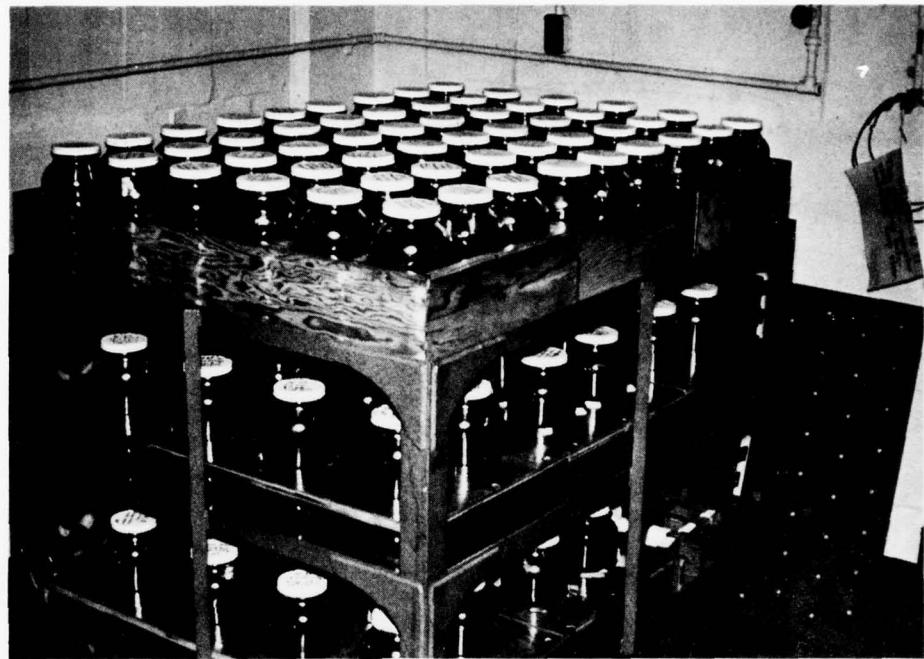


Fig. 1 — Two-phase test units on a rotary shaker

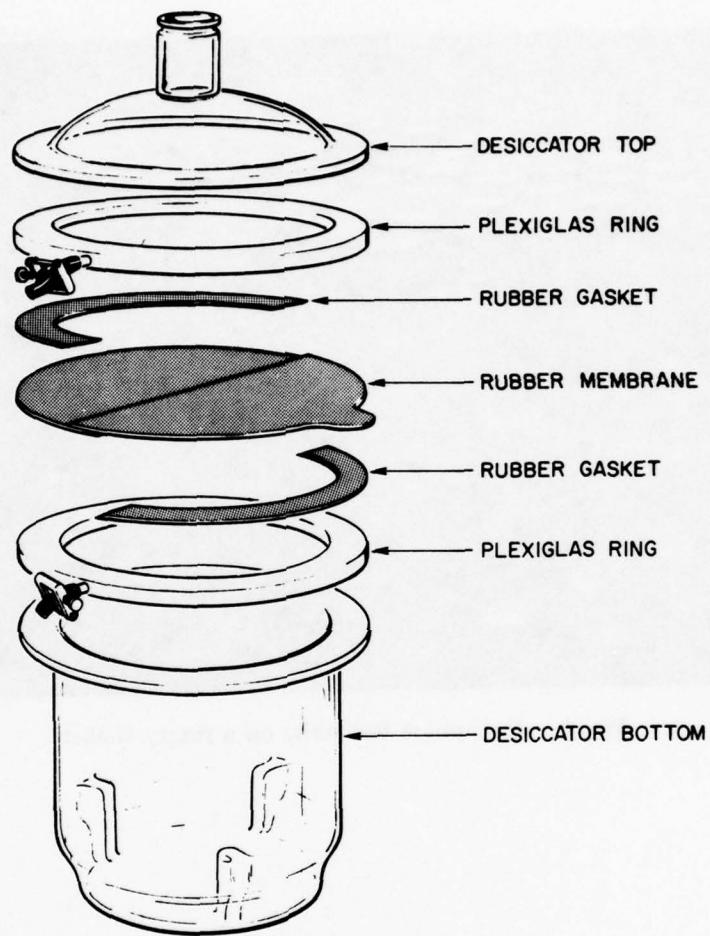


Fig. 2 — Diagram of a membrane separated two-compartment test unit

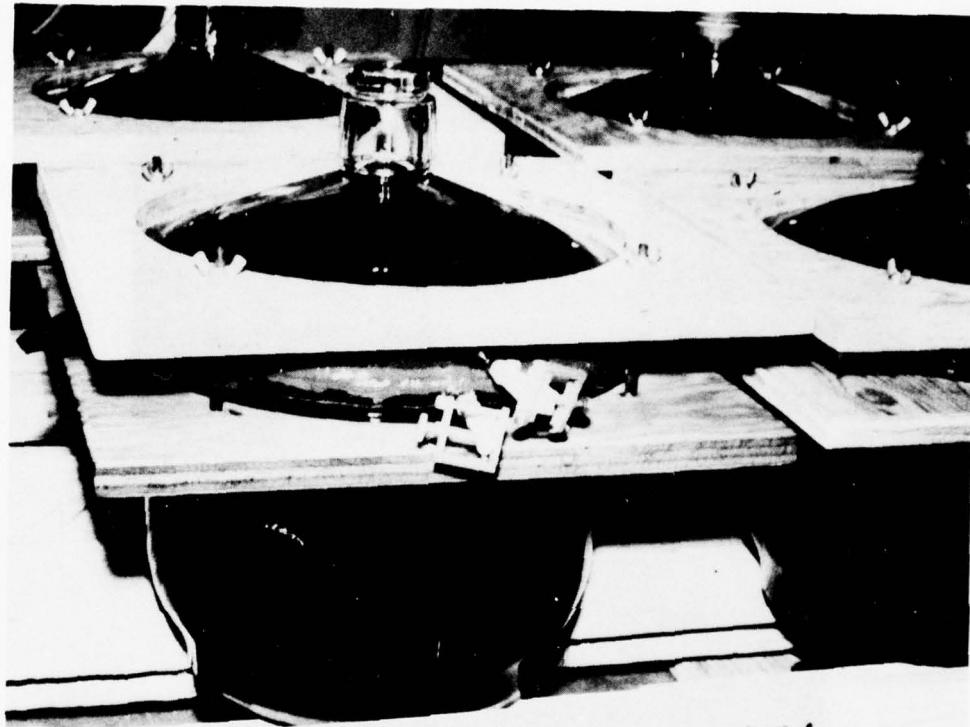
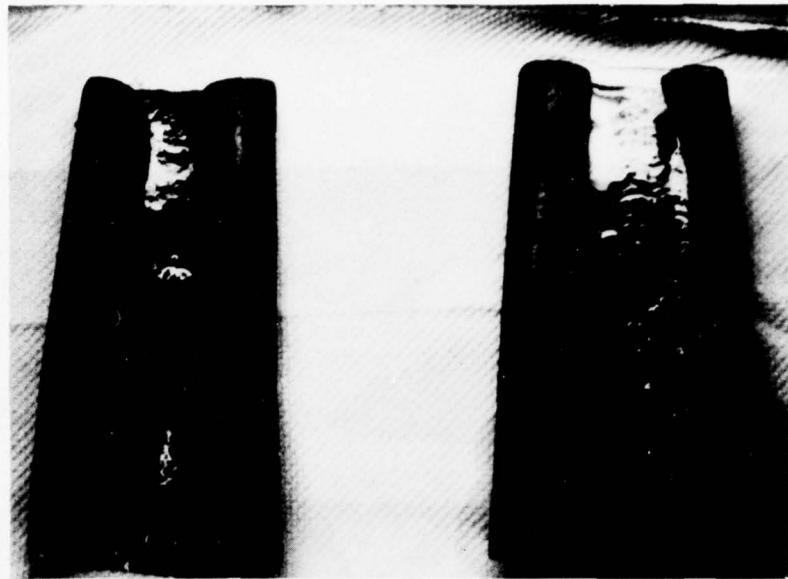


Fig. 3 — Membrane separated two-compartment test unit showing blackening caused by ferrous sulfide

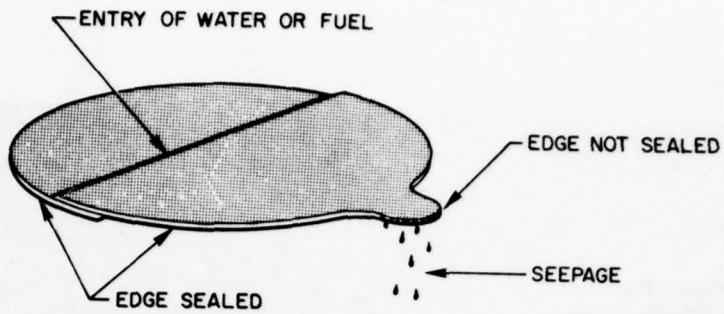


(a) Cold seamed neoprene/nylon cloth/nitrile membranes in a  
JP-5 - sea water system



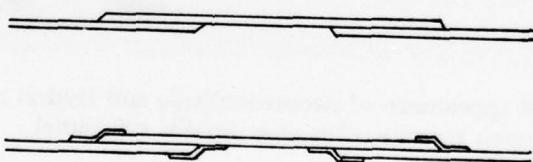
(b) Seamless neoprene/nylon fabric with unidirectional thread/  
nitrile in a JP-5 - sea water system

Fig. 4 — Typical appearance of rubber membranes upon removal from  
the two-phase test units



(a) A separating membrane in a two-compartment test unit

(b) Type of seaming to be avoided when fabricating separator membranes in fuel tanks



(c) Possible fabrication of seams to avoid seepage along fabric in membrane

Fig. 5 — Diagrams depicting the outward diffusion of water or fuel through seam and fabric of rubber membranes

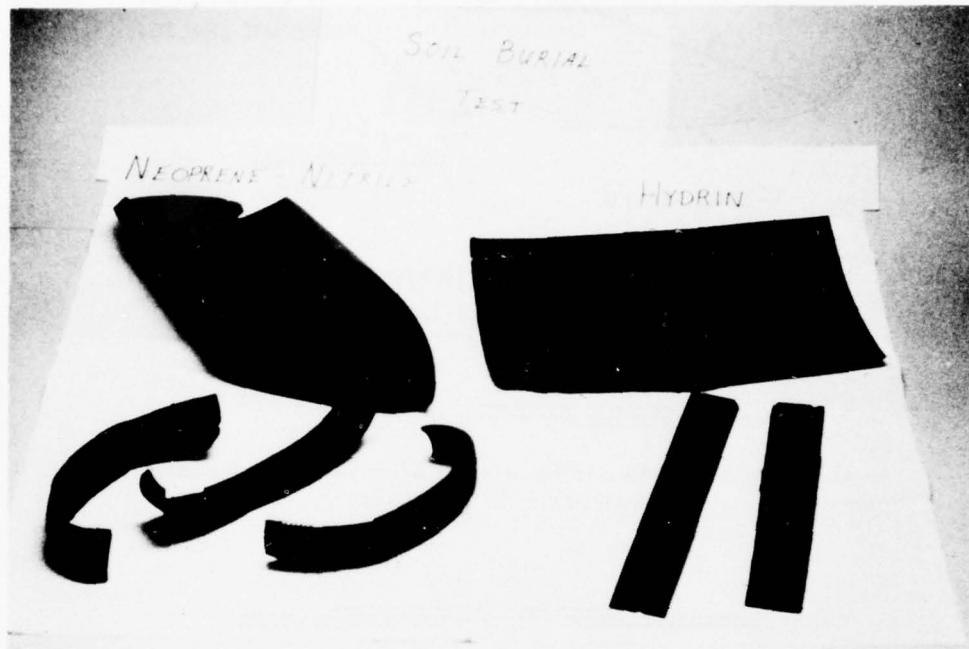


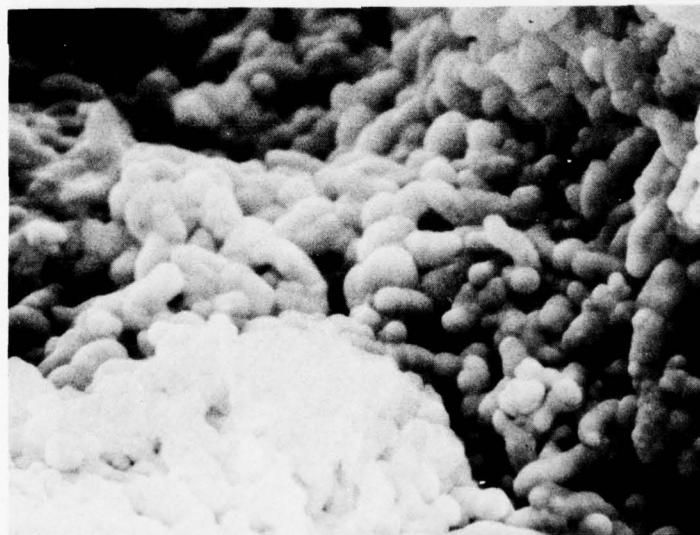
Fig. 6 — Typical appearance of neoprene/nitrile and Hydrin membranes upon removal after nine months soil burial



a

1  $\mu$ m

(a) Fungal material on the surface of a Hydrin membrane after one year exposure in JP-5 - sea water test unit inoculated with selected aerobic microorganisms

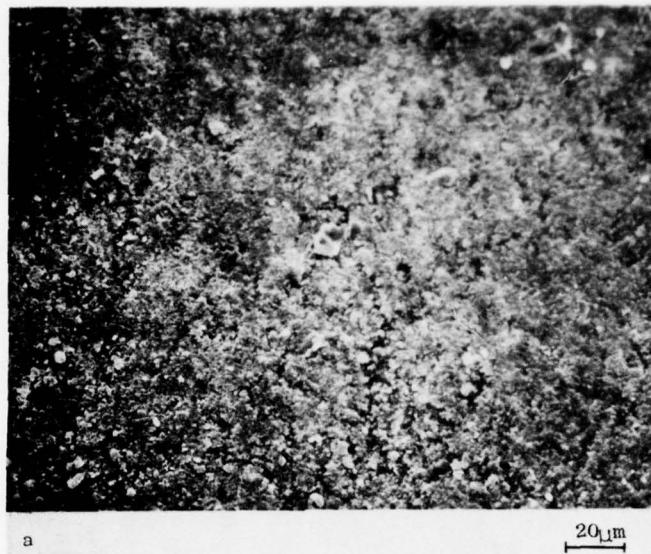


b

1  $\mu$ m

(b) Masses of bacteria on a section of Hydrin membrane at the interface between Navy Distillate and sea water after one year exposure in an anaerobic test unit

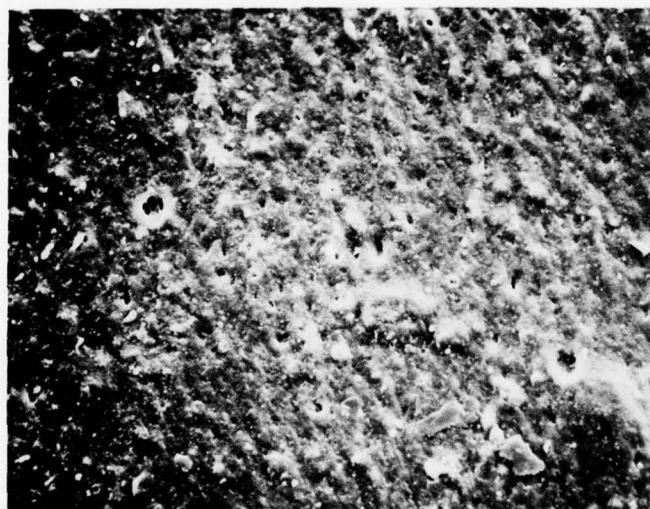
Fig. 7 — SEM photographs of microbial growth on membrane surfaces



a

20  $\mu$ m

(a) Unexposed control



b

20  $\mu$ m

(b) After one year exposure in a D.F.M. - sea water test unit inoculated with an anaerobic culture of sulfate-reducers

**Fig. 8** — SEM photographs of the neoprene side of a bent neoprene/nitrile rubber membrane after detergent cleaning



a

5 μm

(a) Unexposed control



b

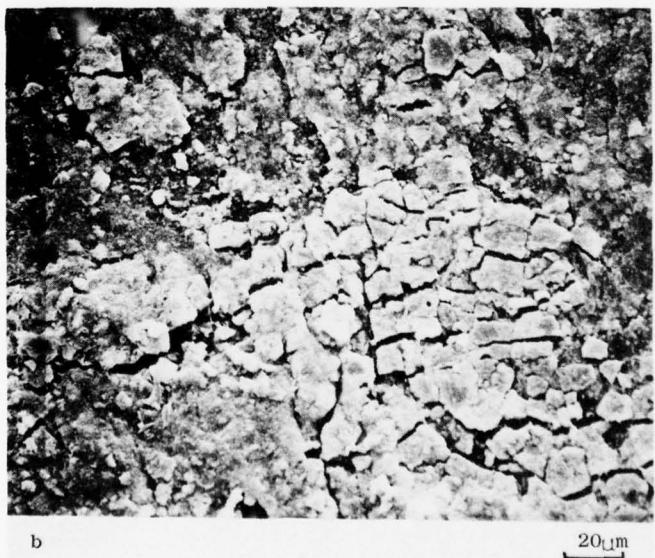
5 μm

(b) After one year exposure in a N.D. - sea water test unit

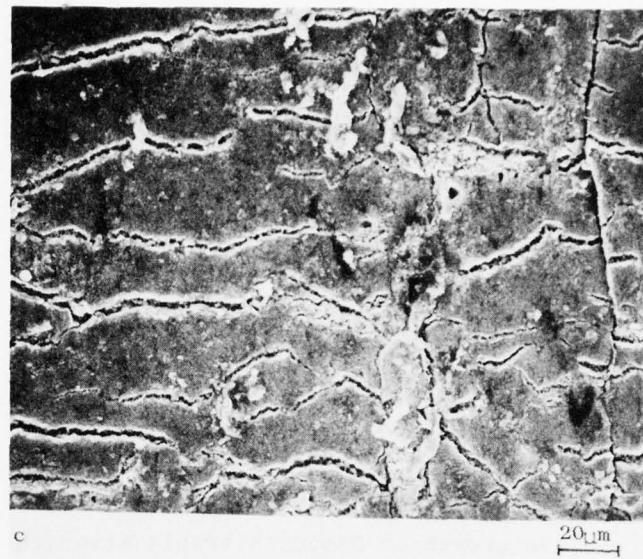
Fig. 9 — SEM photographs of a bent Hydrin membrane after detergent cleaning



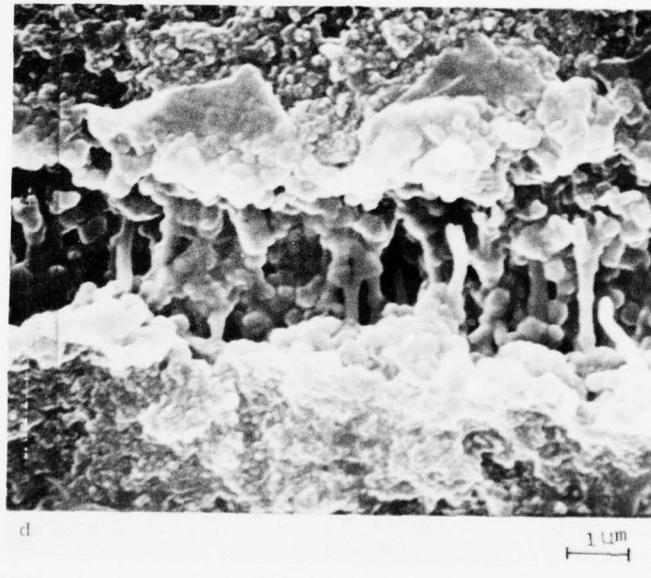
(a) Unexposed control treated with benzene followed by  
a dilute HCl solution



(b) Surface treated with benzene after one year exposure in a  
N.D. - sea water test unit



(c) Surface treated further with dilute HCl solution, which dissolved the flakes, exposing small cracks



(d) Higher magnification of one of the cracks

Fig. 10 — SEM photographs of a bent Hydrin membrane with different surface treatments

Code to Abbreviations - Tables 2a - 10b

D.F.M.	Diesel Fuel Marine
N.D.	Navy Distillate
M.E.C.	Mid-East Crude
I	Inoculated
C	Control, uninoculated
+	Growth on Sisler's triple strength medium
-	No growth on Sisler's triple strength medium

TABLE 1

Growth of Selected Fungi and Bacteria on and around Rubber Strips<sup>1,2</sup>  
on Nutrient-Salts Agar Plates (Room Temperature)

Sterilizing Method	Neoprene-Nitrile			Uniroyal Sealdrum		
	Incubation Time (weeks)			Incubation Time (weeks)		
	1	2	6	1	2	6
Autoclave	2	2	1	1	2	1
Ethylene Oxide	2	2	1	1	1	1
Hypochlorite	2	2	3	2	2	1
Ethanol	1	2	1	1	2	3
Scrub only	1	2	1	1	2	3
No treatment	1	2	1	1	2	3

<sup>1</sup> Observed growth on specimens - Ratings: percent of total surface covered

None	0
Traces of growth (<10%)	1
Light growth (>10%;<30%)	2
Medium growth (>30%;<60%)	3
Heavy growth (>60%)	4

<sup>2</sup> A 1" sq. filter paper control gave a rating of 4 at 1,2 and 6 weeks.

Table 2a  
 Results of Visual Examination of Neoprene-Nitrile Strips (Undirectional Fabric) after Six  
 Months Exposure to Seawater - Fuel. Aerobic Test Units.

Test unit	Examination of surface deposits		Appearance of rubber	Adhesion of rubber to fabric
	Macroscopic	Microscopic		
JP-5	I Heavy white mainly on nitrile side	Bacteria	No change	Good
	C Heavy yellow on neoprene side	Bacteria, coccoid and rod-shaped	No change	Good
D.F.M.	I White on nitrile side	-	No change	Good
	C White on water end of nitrile	-	No change	Good
N.D.	I Small amount of white deposit on water end of nitrile	Bacteria	No change	Good
	C Yellow on nitrile	Bacteria	Neoprene in both phases tenderized	Good
M.E.C.	I White deposit on nitrile side	Bacteria	Neoprene softened, nitrile bleeds oil	Good
	C No deposit	-	No change	Good

Table 2b  
 Results of Visual Examination of Neoprene-Nitrile Strips (Unidirectional Fabric) After Six  
 Months Exposure to Seawater - Fuel. Anaerobic Test Units

Test unit	Surface deposits	Appearance of rubber to fabric	Adhesion of rubber to fabric
JP-5	Some on both sides	No change	Good
	White on both sides	No change	Good
D.F.M.	Some yellow on water end of nitrile, thick and oil soaked in spots	Neoprene tender	Good
	White on both sides	No change	Good
N.D.	White on water end of both sides	No change	Good
	Thin in defined area	No change	Good
M.E.C.	Some white deposit on both sides	No change	Good
	Deposit on nitrile side	No change	Good

Table 3a  
Results of Visual Examination of Neoprene-Nitrile Membranes (Indirectional Fabric) After One  
Year Exposure to Seawater - Fuel. Aerobic Test Units

Test unit	Examination of surface deposits			Appearance of rubber	Adhesion of rubber to fabric
	Macroscopic	Microscopic	Cultures		
I JP-5	Creamy gel at interface and on neoprene	Numerous bacteria, and emulsified oil	Bacteria, fungi	Neoprene tender	Good
	Heavy, orange-yellow on nitrile	Many bacteria	White fungus	Neoprene tender	Good
I D.F.M.	Creamy on neoprene strip	Bacteria, fungal debris	<u>Pseudomonas</u>	Neoprene tender	Good
	Slightly yellow on nitrile	Very few organisms on slide	<u>Pseudomonas</u>	Neoprene tender	Good
I N.D.	On both sides in water phase	Bacteria	<u>Pseudomonas</u>	Neoprene tender	Good
	Orange yellow on nitrile	Many bacteria	<u>Pseudomonas</u> heavy bacterial growth	Neoprene tender	Good
I M.E.C.	Heavy tar at oil-water and oil-air interface	Many bacteria (rods)	<u>Pseudomonas</u>	Neoprene tender	Good
	Hard brown on nitrile water end, tarry crude at interface	Bacteria	Trichoderma other fungi, <u>Pseudomonas</u>	Neoprene tender	Good

Table 3b  
Results of Visual Examination of Neoprene-Nitrile Membranes (Unidirectional Fabric) After  
One Year Exposure to Seawater - Fuel. Anaerobic Test Units

Test unit	Examination of surface deposits			Active sulfate reducers	Appearance of rubber	Adhesion to fabric
	Macroscopic	Microscopic	Cultures			
JP-5	Small amount on neoprene and nitrile in water phase	Bacteria <u>Pseudomonas</u> Bacteria with red soluble pigment	-	-	Neoprene tender	Good
	Creamy on neoprene in water phase, less on nitrile	Numerous bacteria	Sparse growth, large motile rods, Spirilla & small rods	-	Neoprene tender	Good
D.F.M.	Heavy creamy on both sides in both phases	Bacteria	<u>Pseudomonas</u>	-	Neoprene tender	Good
	Thick white jelly-like on neoprene, white on water phase of nitrile	Bacteria & emulsified oil	<u>Pseudomonas</u>	-	Neoprene tender	Good
N.D.	Buff colored on neoprene, at interface. Deposit on, nitrile water phase	Numerous bacteria, other debris	<u>Pseudomonas</u>	-	Neoprene tender (not as much as JP-5 and DFM)	Good
	Buff colored filmy on neoprene and water phase of nitrile	Some bacteria, unidentified debris	Sparse bacteria, white fungus	-	Neoprene tender	Good
M.E.C.	Heavy tar at interface, white bacteria layer between air and nitrile	Bacteria	Bacteria (short motile rods)	-	Neoprene tender	Good
	Some tar deposits creamy bacteria deposits on nitrile	Bacteria	No growth - large motile rods in close association with oil	-	Neoprene tender	Good

Table 4a  
 Results of Visual Examination of Seamless Neoprene - Nitrile Rubber Membranes After One Year Exposure to Seawater - Fuel. Aerobic Test Units

Test unit	Macroscopic	Microscopic	Cultures	Appearance of rubber	Adhesion of rubber to fabric
D.F.M.	Large amount slime in air phase (jar leaked)	Bacteria, fungal material	<u>P. aeruginosa</u> <u>Trichoderma</u>	Curled neoprene tender, finish dulled on sea side	Fair, neoprene pulls away slightly in fuel phase
	Small amount creamy film	Bacteria on section exposed to air	Bacteria	Curled, not tender, finish slightly dulled	Fair, neoprene pulls away slightly in fuel phase
M.E.C.	Moderate amount, tar-like	Bacteria	<u>P. aeruginosa</u> Bacteria	Curled, not tender, finish dulled	Fair, neoprene pulls away slightly in fuel phase
	Small amount, tar-like	Few bacteria	Bacteria	Curled, not tender, finish dulled	Fair, neoprene pulls away slightly in fuel phase

Table 4b  
 Results of Visual Examination of Seamless Neoprene - Nitrile Rubber Membranes After One  
 Year Exposure to Seawater - Fuel. Anaerobic Test Units

Test unit	Examination of surface deposits		Active sulfate reducers	Appearance of rubber	Adhesion of rubber to fabric
	Macroscopic	Microscopic Cultures			
D.F.M.	I	Heavy creamy Emulsion of fuel, bacteria and seawater	Bacteria	+	Curled, neoprene tender, finish dulled
	C	Very little	Bacteria	-	Curled, neoprene slightly tender, finish dulled
M.E.C.	I	Moderate amount, tar-like	Bacteria	+	Curled, not tender, finish slightly dulled
	C	Moderate amount, tar-like	Bacteria	-	Curled, not tender, finish slightly dulled

Table 5a  
Results of Visual Examination of Cold Seamed Neoprene - Nitrile Rubber Membranes After One  
Year Exposure to Seawater - Fuel. Aerobic Test Units

Test unit	Examination of surface deposits			Appearance of rubber	Adhesion of rubber to fabric	Seam adhesion
	Macroscopic	Microscopic	Cultures			
JP-5	I	None	Bacteria <u>P. aeruginosa</u> , <u>Candida</u>	Curled	Fair between fabric and neoprene in fuel	Good
	C	None	Bacteria <u>Pseudomonas</u> sp. bacteria	Curled	Fair between fabric and neoprene in fuel	Good
D.F.M.	I	Very thin film	Bacteria <u>P. aeruginosa</u>	Curled, neoprene slightly tender	Fair between fabric and neoprene in fuel	Good
	C	Thin film	Bacteria, yeast	Curled, neoprene slightly tender	Fair between fabric and neoprene in fuel	Good
N.D.	I	Creamy film in fuel phase	Bacteria	Curled, neoprene tender	Poor between fabric and neoprene in fuel	Good
	C	Moderate orange deposit	Bacteria	Curled, neoprene tender	Poor between fabric and neoprene in fuel	Good
M.E.C.	I	Tar deposit	Bacteria <u>P. aeruginosa</u> , bacteria	Curled, neoprene tender	Fair between fabric and neoprene in fuel	Good
	C	Tar deposit	Bacteria mostly along edge exposed to air	Curled, neoprene tender	Fair between fabric and neoprene in fuel	Good

Table 5b  
Results of Visual Examination of Cold Seamed Neoprene- Nitrile Rubber Membranes After One Year Exposure to Seawater - Fuel. Anaerobic Test Units

Test unit	Examination of surface deposits			Active sulfate reducers	Appearance of rubber	Adhesion of rubber to fabric	Seam adhesion
	Macroscopic	Microscopic	Cultures				
JP-5	Thin creamy at interface and in fuel	Many bacteria	Few bacteria	-	Curled, neoprene tender	Good	Good
	C	Bacteria	Bacteria	-	Curled, neoprene tender	Fair between neoprene and fabric in fuel	Good
D.F.M.	Thin creamy in fuel	Bacteria in deposit	Bacteria	+	Curled, neoprene tender	Fair between neoprene and fabric in fuel	Good
	C	Bacteria	Bacteria	-	Curled, neoprene tender	Fair between neoprene and fabric in fuel	Good
N.D.	Creamy mainly in fuel	Bacteria	Bacteria	-	Curled, neoprene tender	Fair between neoprene and fabric in fuel	Good
	C	Bacteria	Bacteria	-	Curled, neoprene tender	Fair between neoprene and fabric in fuel	Good
M.E.C.	Heavy tar-like at interface	Heavy tar-like at interface	Bacteria	+	Curled, neoprene tender	Fair between neoprene and fabric in fuel	Good
	C	Bacteria	Bacteria	-	Curled, swollen, neoprene tender	Fair between neoprene and fabric in fuel	Good

Table 6a  
Results of Visual Examination of Hot Seamed Neoprene - Nitrile Rubber Membranes After One Year Exposure to Seawater - Fuel. Aerobic Test Units

Test unit	Examination of surface deposits			Appearance of rubber	Adhesion of rubber to fabric	Seam adhesion
	Macroscopic	Microscopic	Cultures			
JP-5	Small amount white in oil phase	Bacteria	<u>P. aeruginosa</u>	Curled, neoprene slightly tender	Poor in fuel phase	Neoprene lifted at seam edge
	None	Few bacteria mostly along edge exposed to air	Bacteria	Curled, neoprene slightly tender	Neoprene pulls away in fuel phase	Good
I	None	Few bacteria	<u>P. aeruginosa</u> , Bacteria	Curled, neoprene slightly tender	Neoprene pulls away in water phase	Good
	D.F.M.	Creamy orange in fuel phase, white film in water phase	Orange deposit, debris, fuel and bacteria, film mainly bacteria	Curled neoprene slightly tender	Poor between fabric and neoprene in fuel	Neoprene lifted at seam edge
I	Very thin film	Bacteria	<u>P. aeruginosa</u> , Bacteria	Curled neoprene tender	Poor between fabric and neoprene in fuel	Neoprene lifted at seam edge
	N.D.	Very thin film	Few bacteria mostly along edge exposed to air(threads)	Bacteria on threads, none on immersed rubber	Curled neoprene tender	Fair at seam edge between neoprene and fabric
I	Moderate tar deposit	Bacteria	<u>P. aeruginosa</u> , Bacteria	Slightly curled, neoprene tender	Poor between fabric and neoprene in fuel	Poor - seam bubbling and lifting between neoprene and fabric in fuel
	M.E.C.	Moderate tar deposit	Bacteria in tar deposit	Bacteria	Curled, neoprene tender	Fair in fuel phase
	C					Fair in fuel phase

Table 6b  
Results of Visual Examination of Hot Seamed Neoprene - Nitrile Rubber Membranes After One Year Exposure to Seawater - Fuel. Anaerobic Test Units

Test unit	Examination of surface deposits			Active sulfate reducers	Appearance of rubber	Adhesion of rubber to fabric	Seam Adhesion
	Macroscopic	Microscopic	Cultures				
JP-5	I Creamy film	Bacteria	Bacteria	+	Curved	Good	Good
C	Very little	No bacteria or fungi observed	Bacteria	-	Curved	Fair, slight separation in corner	
	Very thin creamy film	Few bacteria	Bacteria	+	Curled, neoprene tender	Fair between neoprene and fabric in fuel	Fair, slight separation in corner
D.F.M.	None	No bacteria or fungi observed	No growth	-	Curled, neoprene tender	Fair between neoprene and fabric in fuel	Neoprene separating from fabric in fuel
C							
	Thin creamy in fuel phase	Many bacteria	Bacteria	-	Curled, neoprene tender	Fair between neoprene and fabric in fuel	Fair between neoprene and fabric in fuel
N.D.							
	Creamy in fuel phase	Many bacteria	Bacteria	-	Curled, neoprene tender	Fair between neoprene and fabric in fuel	Good
M.E.C.	I	Very heavy, gummy at interface	Deposit is an emulsion of fuel, bacteria and water	Bacteria	+	Curled, neoprene tender	Fair between neoprene and fabric in fuel
C	Heavy, gummy at interface	Deposit is an emulsion of fuel and water, no bacteria	Bacteria	-	Curled, neoprene tender	Fair between neoprene and fabric in fuel	Good

Table 7a  
Results of Visual Examination of Hydrin Rubber Strips After Six Months Exposure to Seawater-  
Fuel. Aerobic Test Units

Test unit	Examination of surface deposits		Appearance of rubber	Adhesion of rubber to fabric
	Macroscopic	Cultures		
JP-5	Thin and white on both sides	Pseudomonas very active <u>Aspergillus</u> and <u>Trichoderma</u>	Finish on rubber dulled	Good
	Thick and rusty	Many bacteria	Finish on rubber dulled	Good
D.F.M.	Small amount of white deposit in crevices	Pseudomonas, yeasts <u>Trichoderma</u>	Finish dulled	Good
	Thick and rusty	<u>Pencillium funiculosum</u> No growth	Finish dulled	Good
N.D.	White deposit	Pseudomonas, yeasts <u>Fungi-Trichoderma</u>	Finish dulled	Good
	Heavy rusty deposit	No growth	Finish dulled	Good
M.E.C.	Heavy tar deposit in water phase	Pseudomonas, yeasts <u>Trichoderma</u>	Finish dulled	Good
	Heavy tar deposit in water phase	Pseudomonas Salmon-pink bacteria colonies in close association with oil	Finish dulled, roughened	Good

Table 7b  
Results of Visual Examination of Hydrin Rubber Strips After Six Months Exposure to  
Seawater - Fuel. Anaerobic Test Units

Test unit	Examination of surface deposits		Active sulfide reducers	Appearance of rubber	Adhesion of rubber to fabric
	Macroscopic	Cultures			
JP-5	I	No noticeable deposit	Fungus growth	+	Some loss of sheen
	C	Small amount of creamy accumulation	Very little, only 1 bacteria colony	-	Finish dulled
D.F.M.	I	No deposit	No growth	+	Minor loss of sheen
	C	Very little	Thin slime. Slide shows <u>Micrococcus</u>	-	Finish dulled
N.D.	I	No deposit	Fungal colonies	+	Finish slightly dulled
	C	Creamy deposit	White fungus. Yellow bacteria on every spot rubber touched. <u>Micrococcus</u>	-	Finish dulled, roughened
M.E.C.	I	No tar deposit	No growth	+	Finish dulled, sticky
	C	Heavy tar deposits	No growth	-	Finish dulled, sticky

Table 8a  
Results of Visual Examination of Seamless Hydrin Rubber Membranes After One Year Exposure  
to Seawater - Fuel. Aerobic Test Units

Test unit	Examination of surface deposits			Appearance of rubber	Adhesion of rubber to fabric
	Macroscopic	Microscopic	Cultures		
JP-5	Moderate amount, rusty	Bacteria in deposit	<u>P. aeruginosa</u> bacteria	Finish dulled, slightly roughened	Good
	Heavy rusty fungal mat in water phase	Rusty deposit, mainly fungal mycelium	<u>Pencillium</u> sp. <u>Dendryphiella</u> sp. no bacteria	Finish dulled, roughened in water phase	Good
D.F.M.	Light rusty, fungal web	Bacteria Fungi	Bacteria, mainly <u>P. aeruginosa</u> <u>Trichoderma</u>	Finish dulled, roughened in water phase	Good
	Rusty deposit and fungal mat in water phase	No bacteria. Rusty debris attached to fungal mycelium	Fungi	Finish dulled, moderately roughened	Good
N.D.	Fungal web in fuel phase, small amount	Many bacteria, fungal web	<u>P. aeruginosa</u> <u>Trichoderma</u> fungi	Finish dulled, slightly roughened	Good
	Rusty deposit in water phase	No bacteria or fungi	No growth	Very roughened in water phase, roughened in fuel phase	Good
M.E.C.	Tar-like deposit	Bacteria	Bacteria, mainly <u>P. aeruginosa</u>	Finish dulled, slightly roughened	Good
	Tar-like deposit	Few bacteria	Bacteria, mainly <u>P. aeruginosa</u>	Finish dulled, slightly roughened	Good

Table 8b  
Results of Visual Examination of Seamless Hydrin Rubber Membranes After One Year Exposure  
to Seawater - Fuel. Anaerobic Test Units

Test unit	Examination of surface deposits			Active sulfate reducers	Appearance of rubber	Adhesion of rubber to fabric
	Macroscopic	Microscopic	Cultures			
I 1 iridescent in fuel phase	Slight at interface,	Many bacteria	One bacterial colony	+	Finish dulled in water phase, roughened	Good
	iridescent in fuel phase	Bacteria	No growth		-	Finish dulled
JP-5 C at interface	Creamy orange			-	Finish dulled	Good
D.F.M. I fuel phase, no deposit	Iridescent in fuel phase,	Few bacteria	No growth	+	Finish dulled in water phase, very roughened	Good
C in fuel phase	Creamy orange in fuel phase	Bacteria	No growth	-	Finish dulled, slightly roughened	Good
N.D. I No deposit; iridescent in fuel phase	No deposit;	Many bacteria	No growth	+	Finish dulled	Good
	iridescent in fuel phase					
N.D. C Rusty deposit, (unit lost cover - highly contaminated)	Rusty deposit, (unit lost cover - highly contaminated)	Many bacteria	Bacteria	-	Finish dulled	Good
M.E.C. I edge exposed to air	Tar-like on edge exposed to air	Few bacteria	No growth	+	Finish dulled in water phase, slightly roughened	Good
M.E.C. C Tar-like on edge exposed to air	Tar-like on edge exposed to air	Few bacteria	No growth	-	Finish dulled in water phase, slightly roughened	Good

Table 9a  
 Results of Visual Examination of Cold Seamed Hydrin Rubber Membranes After One Year Exposure  
 to Seawater - Fuel. Aerobic Test Units

Test Unit	Examination of surface deposits		Cultures	Appearance of rubber	Adhesion of rubber to fabric	Seam adhesion
	Macroscopic	Microscopic				
JP-5	Light rusty film in water phase	Bacteria	<u>P. aeruginosa</u> bacteria <u>Candida</u> sp.	Slightly roughened	Good	Good
	Small amount rusty deposit	Bacteria at interface	Bacteria	No change	Good	Good
D.F.M.	Rusty film in water phase	Bacteria	Bacteria, mainly <u>P. aeruginosa</u> <u>Trichoderma</u> Fungi	No change	Good	Fair, slight separation
	Slight creamy film in water phase	Bacteria	Bacteria	Slightly roughened	Good	Good
N.D.	Very little	Few bacteria	Bacteria, mainly <u>P. aeruginosa</u> <u>Trichoderma</u> Fungi	Slightly roughened	Good	Fair, slight separation
	Slime on edge exposed to air	Few bacteria	Bacteria	Roughened in water phase	Good	Fair, slight separation
M.E.C.	Moderate amount, tarry	Few bacteria	Bacteria, mainly <u>P. aeruginosa</u>	Roughened	Good	Fair, slight separation
	Small amount, tarry	Few bacteria	Bacteria	Roughened	Good	Fair, slight separation

Table 9b  
Results of Visual Examination of Cold Seamed Hydrin Rubber Membranes After One Year Exposure to Seawater-Fuel. Anaerobic Test Units

Test unit	Examination of surface deposits			Active sulfate reducers	Appearance of rubber	Adhesion of rubber to fabric	Seam adhesion
	Macroscopic	Microscopic	Cultures				
JP-5	I None, iridescent in fuel phase	Bacteria	Bacteria	+	Finish dulled in water phase, roughened	Good	Good
	C Small amount, creamy in fuel phase	Deposit almost totally bacteria	Bacteria	-	Finish dulled, roughened	Good	Good
D.F.M.	I None, iridescent in fuel phase	Bacteria	Fungus	-	Finish dulled in water phase, roughened	Good	Good
	C Very little	Few bacteria	Bacteria	-	Finish dulled, roughened	Good	Good
N.D.	I None, iridescent in fuel phase	Bacteria	<u>Pseudomonas</u> , yeast	+	Finish dulled in water phase, roughened	Good	Good
	C Creamy deposit at interface	Bacteria	Little growth, two bacterial colonies	-	Finish dulled, roughened	Good	Good
M.E.C.	I None	Few bacteria	<u>Pseudomonas</u> , yeast	+	Finish dulled, roughened	Good	Good
	C Small amount, tar-like	Few bacteria	Bacteria, yeast	-	Finish dulled, roughened	Good	Good

Table 10a  
Results of Visual Examination of Hot Seamed Hydrin Rubber Membranes After One Year Exposure  
to Seawater - Fuel. Aerobic Test Units

Test unit	Examination of surface deposits		Appearance of rubber	Adhesion of rubber to fabric	
	Macroscopic	Microscopic		to fabric	Seam adhesion
I	Heavy rusty in both phases	Fungal mat and bacteria in both phases	<i>P. aeruginosa</i> , bacteria, <i>Trichoderma</i> , Yeasts	Slightly roughened	Good
JP-5	Rusty mainly in water phase	Deposit, mainly bacteria	Pink yeast, <i>Cladosporium</i> sp.	No change	Good
C	Creamy film at interface, and many fungal web in fuel phase	Fungal mycelium	<i>P. aeruginosa</i> in bacteria, <i>Trichoderma</i> fungi	No change	Good
D.F.M.	Rusty at interface	Bacteria	Bacteria	No change	Good
C	Very little, film	Fungal mycelium, bacteria	<i>P. aeruginosa</i> , bacteria	No change	Good
N.D.	Creamy film in fuel phase	Bacteria	Bacteria	Slightly roughened in fuel phase	Good
C	Moderate tarry deposit	Few bacteria, few fungal fragments	Bacteria mainly <i>P. aeruginosa</i>	No change	Good
M.E.C.	Small amount, tarry	Bacteria	Bacteria	No change	Good

Results of Visual Examination of Hot Seamed Hydrin Rubber Membranes After One Year Exposure  
to Seawater - Fuel. Anaerobic Test Units

Test unit	Examination of surface deposits			Active sulfate reducers	Appearance of rubber	Adhesion of rubber to fabric	Seam adhesion
	Macroscopic	Microscopic	Cultures				
I JP-5	Small amount black at interface, iridescent in fuel phase	Bacteria	No growth	+	Finish dulled in water phase, slightly roughened	Good	Good
	Creamy yellow at interface	Bacteria	No growth		-	Fair in water phase, slightly roughened	Good
I D.F.M.	Small amount black, iridescent in fuel phase	Many bacteria in black mycelial fragments	No growth	+	Finish dulled in water phase	Good	Good
	Small amount creamy yellow	Many bacteria in deposit	No growth		-	Finish dulled, slightly roughened in water phase	Good
None, N.D.	None, iridescent in fuel phase	Bacteria	No growth	+	Finish dulled, slightly roughened in water phase	Good	Good
	Yellow deposit in water phase	Deposit is an emulsion of bacteria and fuel	No growth		-	Finish dulled, slightly roughened in water phase	Good
I M.E.C.	Small amount tar-like	Few bacteria	No growth	+	Finish dulled, slightly roughened	Good	Good
	Small amount tar-like	Very few bacteria	No growth		-	Finish dulled, slightly roughened	Good

TABLE 11

## Summary of Results of Adhesion of Rubber to Fabric and Seam Adhesion

		Neoprene - Nitrile	Hydrin
Adhesion of rubber to fabric		Fair to poor for neoprene to fabric in fuel phase of all systems Good for nitrile to fabric	Good in all systems
Seam adhesion	Cold seam	Good in all systems	Good in all systems
	Hot seam	Some lifting of neoprene from fabric where seam edges not well sealed	Slight separation in aerobic systems Anaerobic systems good

TABLE 12  
Observations on the membrane separated two-compartment test units after one year's incubation

Sample	Hydrogen sulfide Seawater phase	Hydrogen sulfide Fuel phase	Seawater in fuel phase	Sulfate- reducers	Cultures of 0.1 ml seawater on spread plates***
Hydrin no seam	+	+	-	+	Bacteria
Hydrin cold seam	+	-	-*	**	Bacteria
Hydrin hot seam	+	-	±	+	Bacteria Yeasts
Neoprene/nitrile no seam	+	-	-	+	Bacteria Yeasts Fungi
Neoprene/nitrile cold seam	+	-	-	+	Bacteria
Neoprene/nitrile hot seam	+	-	-	+	Bacteria

\* Small amount  
\*\* Sisler's 3X media took one month to blacken in sample; all other samples blackened in one week  
\*\*\* Media used consisted of PDA+Y, TYG, Marine Agar (Difco 2216)

APPENDIX A  
Formulae of Media

1. Cooke Rose Bengal Agar:\*

Soytone	5.0 g.
Dextrose	10.0 g.
Monopotassium phosphate	1.0 g.
Magnesium sulfate	0.5 g.
Agar	20.0 g.
Rose Bengal	0.035 g.
Distilled water	1000.0 ml.

2. Marine Agar: Bacto-Marine Agar 2216, manufactured by DIFCO  
Laboratories, prepared according the method of Zobell\*

Bacto-Peptone	5.0 g.
Bacto-yeast extract	1.0 g.
Ferric citrate	0.1 g.
Sodium chloride	19.45 g.
Magnesium chloride	8.8 g.
Sodium sulfate	3.24 g.
Calcium chloride	1.8 g.
Potassium chloride	0.55 g.
Sodium bicarbonate	0.16 g.
Potassium bromide	0.08 g.
Strontium chloride	0.034 g.
Boric acid	0.022 g.
Sodium silicate	0.004 g.
Sodium fluoride	0.0024 g.
Ammonium nitrate	0.0016 g.
Disodium phosphate	0.008 g.
Bacto-Agar	15.0 g.
Distilled water	1000.0 ml.

3. PDA+Y: Bacto-Potato Dextrose Agar\*

Potatoes, Infusion from	200 g.
Bacto-Dextrose	20 g.
Bacto-Agar	15 g.
Distilled water	1000 ml.

The above is modified with the addition of

Yeast extract	5 g.
Agar	5 g.

4. Sisler's 3x Medium - Triple Strength Sisler's Medium (10).

Seawater, filtered	1000. g.
DIFCO Agar	3. g.
DIFCO Neopeptone	3. g.
Magnesium sulfate	0.6 g.
Ammonium sulfate	3.0 g.
Sodium sulfite	0.3 g.
Ascorbic acid	0.3 g.
Dipotassium phosphate	0.6 g.
Ferrous ammonium sulfate	0.3 g.
Calcium lactate	10.5 g.

Dissolve agar in seawater by heating, then add remaining ingredients in the order listed. Dispense in 16x125 mm. Screw top test tubes to a height of 75 mm. and autoclave at 15 lb for 15 min. Cool to 50°C; invert tubes repeatedly until solidified; overlay with sterile mineral oil.

5. Trypticase Soy Agar (TSA) - Soybean - Casein Digest Agar, manufactured by BBL, Div. Becton, Dickinson and Co., Cockeysville, Maryland.

Trypticase peptone	15.0 g.
Phytone peptone	5.0 g.
Sodium chloride	5.0 g.
Agar	15.0 g.
Distilled water	1000.0 ml.

6. Tryptone Yeast Glucose Agar (TYG) - Bacto-Plate Count Agar\*

Bacto-Yeast Extract	2.5 g.
Bacto-Tryptone	5.0 g.
Bacto-Dextrose (glucose)	1.0 g.
Bacto-Agar	15.0 g.
Distilled water	1000.0 ml.

\*As presented in DIFCO Supplementary Literature, DIFCO Laboratories, Detroit, Michigan (1966).

## APPENDIX B

### Summary of Early Results on Microbial Inhibition by Neoprene/Nitrile Membranes

During early experiments with a neoprene/nitrile membrane material (sample No. 3 described under Materials and Methods), it was found that some substance leached from the membranes caused considerable inhibition of growth and early die-off of inocula of sulfate-reducing bacteria. The inhibitory substance could not be traced to the method of sterilizing the membrane nor was it due to any of a number of compounding ingredients deemed likely prospects for producing microbial inhibition. The inhibitory substance was not found in any of the subsequently received materials (samples Nos. 4 and 5) nor was it present in Hydrin rubber. Thus the inhibition seems likely to be due to some ingredient or reaction product produced by the curing process which is not always present in neoprene or nitrile formulations. A more detailed description of the methods and results of this part of the investigation may be found in Reference 11.

#### APPENDIX C.

The tables in this appendix consist of physical tests done at the Naval Ship Research and Development Center (DTNSRDC) in Annapolis, Maryland.

Abbreviations used in these tables are as follows:

C = Control  
I = Inoculated  
DFM = Marine Diesel Fuel  
ND = Navy Distillate  
MEC = Mid-East Crude Oil  
PIW = Pounds per inch width

TABLE I  
Tensile Properties of Reinforced Elastomers

Description	Oil	Tensile (ASTM D751)						Tear (ASTM D751)		
		Force at 10% Elong. (PIW)	Force at 30% Elong. (PIW)	Ultimate Force (PIW)	Ultimate Elong. (%)	Energy to Break (1b in <sup>2</sup> )				
Neoprene/Nitrile (original properties)	Untreated	90	450	850	50	175				
Hydrin (original properties)	Untreated	80	440	910	50	195				
		C	I	C	I	C	I	C	I	C
Hydrin	DFM	105	100	515	515	780	875	40	40	130
No Seam	JP-5	100	95	545	470	960	655	45	40	195
Aerobic	ND	100	100	485	550	890	910	45	40	130
	MEC	100	115	515	520	855	855	40	40	170
Hydrin	DFM	100	350	580	600	880	840	40	40	160
No Seam	JP-5	160	450	700	600	940	900	45	40	205
Aerobic	ND	140	120	700	740	920	840	40	40	165
	MEC	120	---	600	---	940	---	45	--	190
Hydrin	DFM	120	120	580	820	860	900	40	40	130
No Seam	JP-5	140	160	620	700	880	820	40	35	160
Aerobic	ND	140	140	600	640	800	900	35	40	110
	MEC	120	120	580	600	800	880	40	40	130

(1 of 2)

Description	Oil	Tensile (ASTM D751)						Tear (ASTM D751)					
		Force at 10% Elong. (PIW)	Force at 30% Elong. (PIW)	Ultimate Force (PIW)	Ultimate Elong. (PIW)	Energy to <sub>2</sub> Break (1b in <sup>-2</sup> )	Tear (1b)	C	I	C	I	C	I
<b>Hydrin</b>													
No Seam	DFM	110	130	525	640	935	995	45	45	185	205	90	100
Anaerobic	JP-5	105	120	560	615	965	980	40	45	220	190	90	90
ND	ND	120	100	580	535	875	870	40	40	170	155	80	65
MEC	MEC	110	110	515	500	905	765	45	40	175	140	100	90
<b>Hydrin</b>													
No Seam	DFM	140	120	600	600	800	820	40	35	115	135	--	--
Anaerobic	JP-5	140	120	600	580	800	820	40	40	120	140	--	--
ND	ND	140	120	620	540	800	860	40	45	135	160	--	--
MEC	MEC	140	65	680	580	820	940	40	45	120	190	--	--
<b>Hydrin</b>													
No Seam	DFM	120	140	720	640	980	850	40	40	180	150	--	--
Anaerobic	JP-5	160	140	700	600	1000	860	45	40	200	165	--	--
ND	ND	140	140	620	600	840	840	40	40	145	140	--	--
MEC	MEC	160	140	660	600	980	880	45	40	200	165	--	--
<b>Neoprene/</b>													
Nitrile No Seam Aerobic	DFM	40	40	180	160	700	670	65	70	210	180	75	75
MEC	MEC	40	40	190	160	680	680	65	70	190	215	75	75
<b>Neoprene/</b>													
Nitrile No Seam Anaerobic	DFM	160	140	500	620	680	660	43	33	198	126	75	75
MEC	MEC	200	140	600	470	980	580	43	33	246	130	75	75

(2 of 2)

Note: All Tensile and Tear are in the Warp Direction.

TABLE IIIa

Adhesion of Fabric to Elastomer  
Peel (180°) Test (ASTM D751)

Exposure description	Oil	Adhesion of Hydrin to fabric (PIW)		
		Hot Seam	Cold Seam	Seamless
Untreated (original properties)		9	6	--
Aerobic control	DFM	6	4	--
	JP-5	4	6	--
	ND	3	6	--
	MEC	7	5	--
Aerobic inoculated	DFM	4	5	--
	JP-5	11	5	--
	ND	4	8	--
	MEC	8	5	--
Anaerobic control	DFM	6	7	--
	JP-5	10	6	--
	ND	5	8	--
	MEC	7	10	--
Anaerobic inoculated	DFM	4	6	--
	JP-5	10	10	--
	ND	7	6	--
	MEC	4	7	--

TABLE IIb

Adhesion of Fabric to Elastomer  
Peel (180°) Test (ASTM D751)

Exposure description	Oil	Nitrile to fabric (PIW)			Adhesion of Neoprene to fabric (PIW)		
		Hot Seam	Cold Seam	Seamless	Hot Seam	Cold Seam	Seamless
Untreated (original properties)		19	--	--	9	--	--
Aerobic control	DFM	15	11	17	3	4	4
	JP-5	25	10	--	5	4	--
	ND	20	13	--	5	4	--
	MEC	24	2	17	5	5	4
Aerobic inoculated	DFM	20	13	14	4	4	4
	JP-5	20	20	--	6	6	--
	ND	30	11	--	4	2	--
	MEC	15	12	--	10	5	--
Anaerobic control	DFM	22	--	14	4	--	3
	JP-5	20	--	--	6	--	--
	ND	16	--	--	4	--	--
	MEC	20	--	15	4	--	4
Anaerobic inoculated	DFM	15	--	17	10	--	4
	JP-5	20	--	--	5	--	--
	ND	20	--	--	13	--	--
	MEC	17	--	18	5	--	4

TABLE III

Adhesion of Fabric to Elastomer  
Membrane Separated Two-Compartment System  
Tests Made Only on Side Facing Oil (DFM)

Seam type	<u>Type of Separation (PIW)</u>	
	Nitrile to fabric	Hydrin to fabric
Hot	30	15
Cold	21	5

TABLE IV  
 Material Properties Before & After Soil Burial \*  
 Tensile & Tear Properties  
 and  
 Elastomer to Fabric Bond  
 ASTM D 751-69 (Strip Method at 12" /min.)

Materials

Orientation	Hydrin/Nylon Cloth/Hydrin				Neoprene/Nylon Cloth/Nitrile			
	Before Warp	Before Fill	After Warp	After Fill	Before Warp	Before Fill	After Warp	After Fill
Tensile Strength (PIW)	971	562	846	663	897	585	960	-
Modulus 10% (PIW)	189	45	172	53	153	35	165	-
Modulus 30% (PIW)	85.9	23.6	63.0	21.5	65.1	16.5	61.8	-
Ultimate Elongation (%)	35	4.9	41	68	39	66	42	-
Tear Strength (lbs.)	137	108	77	-	80	60	68	-
Strain Energy (in. lbs./in <sup>2</sup> )	161	106	193	184	163	158	212	-
Elastomer to Fabric Bond 1bs./in width)	Hydrin before/after				Neoprene before/after			
	7.6/13.0				14.2/23.0			
					6.5/7.7			

\* Method No 5762, CCC-T-191b, 153. Exposure Time = 9 Mo.